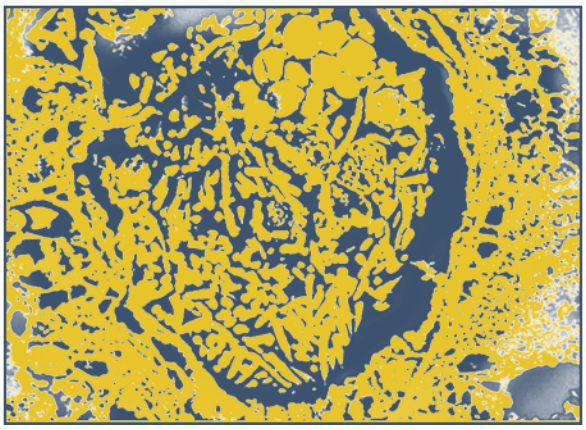
BIODEFENSE

A terrorist attack on the United States using biological agents, once thought to be a remote possibility, occurred in the fall of 2001 when *B. anthracis* spores were sent through the United States mail, causing 18 confirmed cases of anthrax (eleven inhalation, seven cutaneous). Recent events have raised awareness of both the possibility of a bioterrorist attack and the vulnerability of the U.S. population to such an event. In 2003 and 2004, ricin was found in an envelope at a postal facility in South Carolina and a Senate Office Building in Washington, DC, and it was used to contaminate several jars of baby food in California. Although the Department of Defense has developed defenses for biological warfare, there are additional concerns that need to be addressed to provide an adequate civilian defense from a bioterrorist attack. The number of microbial pathogens that threaten civilian populations is larger than that of classical biological warfare threats. Moreover, the populations to be protected are different because civilians include people of all ages and physical conditions.

In 2002, NIAID developed a strategic plan for biodefense research that outlines plans for addressing research needs for bioterrorism and emerging and re-emerging infectious diseases. In addition, NIAID convened a Blue Ribbon Panel of experts to provide objective scientific advice on NIAID's biodefense research agenda on so-called Category A agents. This list, which is defined by the Centers for Disease Control and Prevention, includes the most dangerous threat agents, such as smallpox and anthrax. The expert panel was asked to assess the current research, identify goals for the highest-priority areas, and make recommendations to achieve the goals. In the fall of 2002, NIAID convened a similar expert panel to assess current research and identify goals for Category B and C agents. In the areas of immunology and biodefense, NIAID has convened two more advisory bodies:



***Bacillus anthracis*, the rod-shaped organism that causes anthrax.**

an Expert Panel on Immunity and Biodefense, to assess future immunology research most important to combat bioterrorism and emerging infectious diseases; and an Expert Panel on Atopic Dermatitis and Vaccinia Immunization, to develop a research plan to reduce the risk of eczema vaccinatum, a serious and sometimes deadly complication of smallpox immunization in atopic dermatitis patients.

In the past year, NIAID has continued to expand, intensify, and accelerate its ongoing research programs in biodefense. NIAID has launched research initiatives in areas ranging from the basic biology of microbes and their interactions with the human immune system to preclinical and clinical evaluation of new therapeutics and vaccines. These initiatives are designed to take advantage of the recent outpouring of ideas from academic and industrial scientists on ways to understand and combat potential agents of bioterrorism (*www2.niaid.nih.gov/biodefense*). In addition, NIAID released two progress reports highlighting accomplishments in biodefense research during the 18 months subsequent to the development of the strategic plan (*www.niaid.nih.gov/biodefense/research/category_a_progress_report.pdf; www.niaid.nih.gov/biodefense/research/category_bc_progress_report.pdf*).

Basic Research

One of the most important basic research tools that has evolved in recent years is the ability to rapidly sequence the entire genomes of microbial pathogens, including potential agents of bioterrorism. This capability allows scientists to identify microbial genes that play a role in disease and then design drugs that can block the activities of the proteins encoded by these genes. NIAID has made a significant investment in the DNA sequencing of the genomes of microorganisms considered agents of bioterrorism, including several Category A, B, and C agents. Organisms NIAID has helped to sequence include *Brucella suis, Burkholderia mallei, Clostridium perfringens, Coxiella burnetii, Rickettsia typhi, Staphylococcus aureus, Yersinia pestis, Mycobacterium tuberculosis, Vibrio cholerae, Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica, Toxoplasma gondii,* diarrheagenic *Escherichia coli, Shigella,* and *Salmonella.* In addition, NIAID has expanded its sequencing efforts of *B. anthracis* beyond the Ames strain used in the 2001 attack and has developed a comprehensive genomic analysis that includes sequencing of at least 14 additional strains, clinical isolates, near neighbors, and related species. These sequences will facilitate forensic strain identification; understanding of microbial pathogenesis; discovery of new targets for drugs, vaccines, and molecular signatures; and discovery of biomarkers for diagnostics to combat bioterrorism.

To expand its current enteric pathogens research network, NIAID established the Food and Waterborne Diseases Integrated Research Network to include multidisciplinary research on all food- and water-borne pathogens or toxins. The network also will facilitate the development and evaluation of products to rapidly identify, prevent, and treat food- and water-borne diseases that threaten public health.

Immunity and Biodefense

Considerable knowledge about the mechanisms of host immune responses to microbial pathogens has been gained in recent years. Studies of innate immune mechanisms, which serve as a nonspecific first line of defense against pathogenic infection, have been especially productive. For example, the identification and functional characterization of the Toll-like family of receptors expressed on cells that mediate human innate immunity have led to an explosion of information now being applied to the development of new vaccine adjuvants and immunostimulatory agents to boost nonspecific immune protection. Additional progress on understanding the molecular mechanisms responsible for pathogen-specific immunity mediated by antibodies and cytotoxic T cells has led to new approaches in vaccine design. For example, NIAID-sponsored scientists identified two short peptides from vaccinia—the benign virus used as a smallpox vaccine—that are recognized by the immune systems of people who have been immunized. Researchers can use these peptides to track the human immune response to the virus as they try to develop an improved vaccine. Finally, the threat of bioterrorism and the natural emergence of diseases due to microbes such as West Nile virus and severe acute respiratory syndrome (SARS) virus underscore the importance of defining the immune parameters responsible for increased susceptibility to infectious diseases of infants, young children, the elderly, and immunocompromised individuals.

To gain a better understanding of the human immune response to potential agents of bioterror, NIAID funded eight Cooperative Centers for Translational Research on Human Immunology and Biodefense. These centers, located throughout the country, focus on rapid development of bioterrorism countermeasures, such as vaccines and therapies.

Also contributing to the biodefense vaccine effort are a number of recent contracts awarded to identify immune epitopes for Category A, B, and C pathogens; define human genetic variance that contributes to infection susceptibility or vaccine efficacy; identify new candidates for

vaccine adjuvants; develop reagents for nonhuman primate studies of new drug or vaccine candidates; and address the problem of eczema vaccinatum as a serious adverse consequence of the current smallpox vaccine.

New Diagnostic Tools

NIAID also supports research leading to the development of new and improved diagnostics. The goals of this research are to establish methods for the rapid, sensitive, and specific identification of natural and bioengineered microbes, as well as to determine the microbes' sensitivity to drug therapy. Progress in these areas will allow healthcare workers to diagnose and treat patients more accurately and quickly.

In FY 2004, NIAID developed two initiatives that specifically support the development of the next generation of medical diagnostics—Challenge Grants: Biodefense and SARS Product Development; and Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense and SARS. Under these initiatives, 45 awards were made in FY 2004. NIAID also continues to support its Small Business Biodefense Program, which encourages the development of therapeutics, vaccines, adjuvants/immunostimulants, diagnostics, and selected resources for biodefense by the small business research community. This program expands the duration and dollar limits for small business grants to develop specified products that are considered high priority for biodefense.

NIAID supports a range of biodefense genomics research projects that provide comprehensive genomic, bioinformatics, functional genomics, and proteomic research resources to the scientific community to help researchers identify targets and proteins for use in new diagnostics. Through these projects, NIAID awarded contracts in FY 2004 for eight Bioinformatics Resource Centers to develop and maintain comprehensive, relational databases for genomic and related data for microorganisms responsible for emerging and re-emerging infectious diseases and for those considered agents of bioterrorism (*www.niaid.nih.gov/dmid/genomes/brc/default.htm*). NIAID also awarded contracts for seven Biodefense Proteomics Research Centers and one coordinating center to develop and enhance innovative proteomic technologies and methodologies and apply them to the understanding of the pathogen and/or host cell proteome for the discovery and identification of novel targets for the next generation of drugs, vaccines, diagnostics, and immunotherapeutics against microorganisms considered agents of bioterrorism (*www.niaid.nih.gov/dmid/genomes/prc/default.htm*). In addition, the NIAID-supported Pathogen Functional Genomics Resource Center was expanded to provide the infectious disease research community with state-of-the-art research and reference reagents that can be used in the development of diagnostics or other products.

Vaccines

NIH-supported researchers are developing vaccines against many infectious agents, including those considered to be bioterrorism threats, for use in civilian populations of varying ages and health status. Vaccines are being developed using both traditional and novel technologies. Significant progress has been made in the development of next-generation vaccines for anthrax and smallpox, and in the development of new vaccines for Ebola and West Nile viruses.

In 2003, NIAID awarded four contracts to fund development of new vaccines against smallpox, plague, and tularemia. The smallpox contract awards continue advanced development work that began in February 2003 on two modified vaccinia Ankara (MVA) vaccine candidates. These new contracts will support larger-scale manufacturing of the vaccines, as well as further safety and efficacy studies in animals and humans. The tularemia and plague contract awards will fund early-stage product development of the respective

vaccines, including dosage formulation, pilot batch production, and initial clinical assessment.

Therapeutics

NIH therapeutics research focuses on the development of new antimicrobials and antitoxins, as well as the screening of existing antimicrobial agents to determine whether they have activity against organisms that might be used by bioterrorists. Knowledge gained from basic and applied research is helping to identify additional targets for medications and immune-based therapies against agents of bioterrorism.

In FY 2004, NIAID made additional awards to expand the resource pool in the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense Program to provide a range of resources for preclinical testing of new therapies and vaccines, including nonhuman primate models.

NIAID also has expanded the Collaborative Antiviral Study Group (CASG) by approximately 20 percent since it was established in 1986. In 2003, CASG developed a clinical protocol for the treatment of smallpox with cidofovir, in the event of an outbreak or release. NIAID is currently supporting a phase I clinical study by Chimerix, Inc. to assess initial safety, tolerability, and pharmacokinetics of a promising new oral derivative of cidofovir in normal volunteers. The CASG will conduct future phase I/II studies with the drug after the initial phase I study is complete.

Research Resources

Over the past year, NIAID has continued to devote considerable resources to the expansion of centralized laboratory resources, including regional biosafety laboratories, *in vivo* and animal model resources, drug screening contracts, the production of standardized and validated reagents and tests, and genomic and bioinformatics resources. The availability of such resources assists the research community in conducting studies of biodefense pathogens.

Biodefense and Emerging Infections Research Resources Repository

NIAID established the Biodefense and Emerging Infections Research Resources Repository in September 2003 to provide unique and quality-assured biodefense-related reagents and resources to the scientific community. This program helps facilitate the understanding of the pathogenesis of NIAID category A, B, and C priority pathogens and emerging infectious diseases organisms, and may aid in the development and evaluation of vaccines, therapeutics, and diagnostics for these organisms. The repository also coordinates access to reagents not held in the program.

In order to facilitate research and product development for biodefense and emerging infectious diseases, the repository is collecting information about biodefense-related reagents and standards and will disseminate this information through print, electronic media, and workshops; enhance technology transfer through development and publication of methods; and facilitate commercial development of reagents through proactive communication with biotechnology and pharmaceutical companies. In addition to securing acquisition, storage, and the distribution of biological agents, the repository will also generate new reagents as scientific advances are made.

It is anticipated that in the long-term the Biodefense and Emerging Infections Research Resources Repository will become the Federal government's national resource and clearinghouse for specimens, reagents, and information on these organisms. By centralizing this function, access to and use of these materials can be monitored and quality control of the reagents assured. Information about this resource is now available on the Web site at *www.beiresources.org*.

NIAID Intramural Research Programs

Biology of the Microbe

The NIAID Division of Intramural Research (DIR) studies of *B. anthracis*, the bacterium that causes anthrax, are focused on identification, genetic regulation, and analysis of the anthrax toxin and other virulence factors, as well as development of improved vaccines and therapeutics. The anthrax toxin is the primary cause of damage to animal tissues during an anthrax infection. Recent NIAID intramural studies of anthrax toxin aim to identify organs, tissues, cells, and proteins targeted by anthrax lethal toxin; characterize the contribution of anthrax edema toxin and capsule to pathogenesis; and define the molecular details of the interaction of anthrax toxin with its receptors. In addition, scientists plan to identify changes in the proteome of tissues damaged by anthrax toxin and measure the ability of anti-capsule antibodies to protect against anthrax infection.

NIAID intramural investigations of *Yersinia pestis*, the bacterium that causes plague, have resulted in the development of both mouse and rat models of bubonic plague that incorporate the natural flea-borne route of transmission. A new plague vaccine candidate developed by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) was recently found to be 100 percent effective in the NIAID mouse model.[19] This work is important to NIAID's biodefense efforts as well as to efforts to control naturally occurring plague epidemics. In light of recent plague outbreaks in human populations in India and Africa and the emergence of multiple antibiotic-resistant strains of *Y. pestis*, plague remains an international public health concern.

To better understand the innate immune response, DIR scientists are studying infection-fighting white blood cells called neutrophils, which are an essential part of human innate immunity. Although much is understood about the innate immune response to infection, the molecular basis for termination of inflammation and resolution of infection in humans is not clearly understood. To that end, NIAID researchers have produced a comprehensive new picture of the interactions between many kinds of disease-causing bacteria and neutrophils. By describing changes in neutrophil gene expression in response to bacterial invasion, the investigators have identified dozens of possible targets for drug therapies. These findings are likely to be broadly applicable to many types of microorganisms that cause disease in humans, and could lead to new treatments that augment the immune response against multiple high-priority pathogens.[20]

Additional investigations underway in NIAID laboratories include studies of the pathogenesis of *C. burnetii*, the agent of Q fever; studies of multidrug-resistant tuberculosis; studies of relapsing fever agents with a focus on improving diagnostic tests; and a new program to identify and characterize antigens suitable for use in a vaccine against *Burkholderia mallei* and *Burkholderia pseudomallei*, the causative agents of glanders and melioidosis, respectively. This research is supported by enhanced genomics and proteomics capabilities on the Bethesda campus and at the Rocky Mountain labs.

Vaccines

NIAID has a longstanding intramural research program aimed at shedding light on the molecular biology and gene expression mechanisms used by vaccinia—the virus used in the current smallpox vaccine—and other poxviruses. A primary aim of this program is the development of MVA as a carrier for the delivery of vaccine components and gene therapies to target cells. Intramural poxvirus researchers, who have decades of experience with MVA, and other poxvirus scientists are collaborating with USAMRIID researchers and others in nonhuman primate studies of MVA's efficacy as a smallpox vaccine. In a study comparing MVA and Dryvax in a monkey model, scientists found that after two doses of MVA or one MVA dose followed by

Dryvax, the immune response was equivalent or higher than that induced by Dryvax alone. After challenge with monkeypox virus, unimmunized animals developed hundreds of skin lesions and became gravely ill or died, whereas vaccinated animals were healthy and asymptomatic, except for a small number of transient skin lesions in animals immunized only with MVA. These findings are important steps in the evaluation of MVA as a replacement vaccine or pre-vaccine for those with increased risk of severe side effects from Dryvax.[21]

In addition, researchers at the Vaccine Research Center (VRC) are working to complete two phase I clinical trials in which MVA is evaluated in both vaccinia-naive (never vaccinated) and vaccinia-immune (previously vaccinated against smallpox) populations.

Hemorrhagic fevers such as those caused by Ebola virus are associated with a high mortality rate, particularly for the Ebola Zaire subtype. Traditional public health measures to prevent future outbreaks are limited, thus increasing the urgency for development of an effective vaccine. An interagency agreement currently in place between NIAID and USAMRIID allows for collaboration in animal studies, assay performance, and data analysis.

A potentially effective adenoviral vector-based (ADV) vaccine for Ebola virus infection in nonhuman primates has been developed under an interagency agreement between NIAID and USAMRIID. An ADV-only vaccine that elicited protective immunity in monkeys after a 4-week postvaccination challenge, in contrast to previous 10-week or 6-month vaccine regimens, could be especially useful in an acute Ebola outbreak, if the vaccine proves as effective in humans. A second-generation product may also be evaluated that could potentially provide coverage for Marburg and possibly Lassa viruses. In addition, the VRC began a phase I trial of a DNA-based vaccine for Ebola in November 2003.

The VRC is currently conducting preclinical testing of a West Nile virus vaccine. The VRC proposes to use an existing codon-modified gene-based DNA plasmid vaccine platform to make DNA constructs that express West Nile virus proteins. These vaccine constructs are currently undergoing immunogenicity and viral challenge studies in rabbits. The VRC, in collaboration with Vical, Inc., has completed good manufacturing practices production of the vaccine for a phase I trial scheduled for early 2005.

Therapeutics

NIAID clinical investigators have an approved protocol in place that will allow them to evaluate and treat persons exposed to or infected with anthrax and to conduct immunologic evaluations of recipients of anthrax vaccines. In addition, DIR investigators and their colleagues in the NIH Clinical Center are collecting serial blood samples and throat swabs from healthy persons who receive the smallpox vaccine in order to measure serum cytokines and look for the smallpox vaccine virus. Identification of specific cytokines induced after vaccination may help to explain certain side effects associated with the smallpox vaccine and suggest new ways to modify some of these side effects. Investigators also are evaluating different methods of detecting the smallpox vaccine virus in clinical specimens, including sensitive cell culture methods and polymerase chain reaction.

Protective antibodies are produced by the host in response to infection or immunization. Administration of sera containing protective antibodies to people exposed to a pathogen is called passive immunoprophylaxis and has long been used to prevent disease in exposed populations. However, monoclonal immunoglobulin preparations tailored to act specifically on the most vulnerable parts of an invading pathogen could be of higher and more consistent potency.

DIR researchers are pursuing several prophylaxis and treatment strategies based on monoclonal antibodies, including the development of preparations that can be used to prevent or treat complications of smallpox vaccination, smallpox, anthrax, SARS, West Nile virus, botulism, rabies virus, Japanese encephalitis virus, and the tick-borne encephalitis virus complex. For example, DIR researchers are developing preparations of monoclonal antibodies from chimpanzees—which are virtually identical to human antibodies—that can bind specific antigens on the vaccinia virus and might therefore be used in treatment of complications arising from the use of this virus as a smallpox vaccine.